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A1

WO 02/39835

(54) Title: INDIGESTIBLE OLIGOSACCHARIDES AND MAGNESIUM ABSORPTION IN HUMANS

(57) Abstract: The present invention relates to a composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium. The indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomaltosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof. Furthermore, the use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans is disclosed.

Indigestible oligosaccharides and magnesium absorption in humans

Technical field

The present invention is in the field of human nutrition and health. Specifically it relates to a method of increasing absorption and bioavailability of magnesium. Indigestible oligosaccharides are shown to have this effect when added in effective amounts to normal food.

Background of the invention

Magnesium is an essential dietary element that plays an important role in the body. It is acting as a cofactor in many enzymatic reactions, including glucose use, the synthesis of fat, proteins and nucleic acids, the metabolism of adenosine triphosphate, muscle contraction and some membrane transport systems. It is active in all the major metabolic processes involved in the body's defences, stimulating growth and immunity. It has multiple effects and properties like anti-stress, antiallergenic, anti-inflammatory, radioprotective, and acting as a thermal regulator.

Tucker K. et. al in Journal of Bone Mineral Research 10, (1995), S466 shows the role of magnesium in the prevention of osteoporosis by diminution of bone. Durlach J. et. al in Magnesium Research 12, 1. (1999) pages 57-61 demonstrates the importance of magnesium in the reduction of risk of coronary disease. Similarly, Mizushima S., et. al. in J. Hum. Hypertens. 12(7). (1998) pages 447-53 shows that magnesium is involved in the reduction of hypertension. Furthermore, Djurhuus MS, et.al. in Diabetes Care, Apr. 22(4) (1999) pages 546-54 demonstrates that magnesium is involved in the body's ability to secrete insulin and to reduce insulin resistance in diabetics.

T. Sako et al. in International Dairy Journal 9 (1999), pages 69-80 shows that fructans such as inulin and fructo-oligosaccharides effect calcium and magnesium absorption in the rat intestine. However, it is further indicated that these results are not supported by human studies. In fact, up to now the contrary has been proven and compositions of galacto-oligosaccharides, inuline, or fructo-oligosaccharides do not lead to improvement in calcium or iron absorption in young male volunteers.

C.J. Ziemer et. al. in Int. Dairy Journal 8 (1998) pages 473 – 479 describes that fructo-oligosaccharides are able to modify the gut flora composition in favour of bifidobacteria.

E. van den Heuvel et. al. in Am. J. Clin. Nutr. 1998; 67: 445-451 describes that inuline, fructo-oligosaccharides, or galacto-oligosaccharides do not have a negative effect on iron and calcium absorption in young healthy men. However, no positive indication nor any effect on magnesium absorption is disclosed.

M. D. Collins et al. in Am. J. Clin. Nutr. 1999; 69(suppl.): 1052S-1057S provides an overview of how probiotics, prebiotics, and synbiotics may contribute toward nutritional modulation of the gut microecology. However, beneficial aspects of human gut flora still need definitive confirmation.

Currently, in the United States and in Europe, magnesium supplied by food is often below the recommendations. Whereas the recommended intake corresponds to around 6 mg per kg per day, the normal daily intake is only in the order of 4 mg per kg. A large proportion of the population therefore presents a primary, chronic magnesium deficiency, through nutritional insufficiency. Such a chronic magnesium deficiency can result in chronic fatigue, signs of neuromuscular hyper-excitability analogous to those described under latent tetany and hyperventilation syndrome.

Accordingly, there exists a need for improving the nutritional intake of magnesium.

The current invention provides a composition for increasing the absorption and/or bioavailability of magnesium.

Summary of the invention

The present invention relates to a composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium.

The present invention relates to a composition wherein the functional ingredient is present in an amount of between 1% w/w to 99% w/w, preferably between 2% w/w to 90% w/w, more preferably between 5% w/w to 80% w/w, most preferably between 20% w/w to 50% w/w.

The present invention further relates to a composition wherein the functional ingredient is enriched with 1% w/w to 20% w/w magnesium salt, preferably between 3% w/w and 10% w/w magnesium salt.

The composition of the current invention is further characterised in that the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

The present invention further relates to the use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans.

The present invention further relates to the use wherein the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

Furthermore, the present invention relates to the use wherein the indigestible oligosaccharide is administered in a daily dose of between 1 to 20 g/kg body weight, preferably between 2 to 17 g/kg body weight, more preferably between 5 to 15 g/kg body weight.

The present invention relates to the use of indigestible oligosaccharide in the preparation of a food product for increasing absorption and/or bioavailability of magnesium.

The present invention relates to a food product containing aforementioned composition and said food product is selected from the group consisting of bakery products, snacks, breakfast cereals, cereal bars, dairy products, desserts, confectionery products, dietary supplements and beverages.

The present invention relates to the use of indigestible oligosaccharide in the preparation of a pharmaceutical product for increasing absorption and/or bioavailability of magnesium.

Furthermore, the present invention describes a pharmaceutical product comprising the aforementioned composition and said pharmaceutical product is provided with a carrier selected from the group consisting of tablets, lozenges, capsules, suspensions and syrups.

Detailed description of the invention

The present invention relates to a composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium.

So far, it has been demonstrated that in rat intestine, fructans, inulin and fructo-oligosaccharides effect magnesium absorption. However, rats have a greater cecum and a different food matrix than humans and the results related to improved magnesium absorption cannot be transposed straightforward from rats to humans. In fact, it has been indicated that inulin, fructo-oligosaccharides, or galacto-oligosaccharides do not have a negative effect on iron and calcium absorption in young healthy men (E. van den Heuvel et. al. in Am. J. Clin. Nutr. 1998; 67: 445-451). However, no positive indication nor any effect on magnesium absorption is disclosed.

Surprisingly, the current invention demonstrates that an effective amount of indigestible oligosaccharide increases in humans the absorption and/or bioavailability of magnesium.

The effective amount of the functional ingredient is the dose, which is sufficient for obtaining an improved absorption and/or bioavailability of magnesium in humans.

The absorption of magnesium can be measured by stable magnesium isotope analysis with inductive coupled plasma mass spectrometer (ICP/MS).

Indigestible oligosaccharide refers to a carbohydrate moiety that is resistant to endogenous digestion in the human upper digestive tract.

The present invention relates to a composition wherein the functional ingredient is present in an amount of between 1% w/w to 99% w/w, preferably between 2% w/w to 90% w/w, more preferably between 5% w/w to 80% w/w, most preferably between 20% w/w to 50% w/w.

The composition of the current invention is further characterised in that the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides or isomalto-oligosaccharides.

Xylo-oligosaccharides are produced by the enzymic hydrolysis from xylan, which is one of the main components of dietary fibre, and it is a major component of plant hemicellulose.

Galacto-oligosaccharides comprise di-, tri-, tetra-, penta- and hexasaccharides, mainly consisting of galactose as a sugar component, and are formed by the action of β -galactosidase on lactose.

Soybean oligosaccharides are the water-soluble saccharides extracted from soybean whey, a by-product from the production of soy protein. Mainly mixtures of mono-, di-, tri-, and tetrasaccharides with as principal components raffinose and stachyose are obtained.

Gentio-oligosaccharides are polymers in which 6- β -glucopyranosyl-glucose constitute the majority of linkages.

Isomalto-oligosaccharides are mixtures of isomaltose, panose, isomaltotriose, and several other branched oligosaccharides composed of four and five glucose residues.

Fructans are polymers in which fructosyl-fructose linkages constitute the majority of linkages.

Fructo-oligosaccharides are indigestible oligosaccharides that are members of the inuline subclass of fructans. Fructo-oligosaccharides occur in nature in many kinds of plants, including onions, garlic, shallots, wheat, rye, bananas, aspargus, tomatoes, artichokes, dahlia and chicory root. Fructo-oligosaccharides can be produced enzymatically, through chemical techniques or by extraction from natural substances. Short chain fructo-oligosaccharides are composed of one to three fructose molecules linked to one molecule of sucrose. Their polymerisation degree (DP) is not higher than 6, and they can be synthesised from sucrose through the use of transfructosylating enzymes. Treatment of sucrose with these transfructosylating enzymes results in a mixture of

fructo-oligosaccharides containing 2, 3 or 4 fructose units, such as 1-kestose, nystose, and fructosyl-nystose.

The current invention surprisingly demonstrates that an effective amount of these short chain fructo-oligosaccharides increases in humans the absorption and/or bioavailability of magnesium.

The present invention further relates to a composition wherein the functional ingredient is enriched with 1% w/w to 20% w/w magnesium salt, preferably between 3% w/w and 10% w/w magnesium salt.

The magnesium salt can be magnesium carbonate, magnesium citrate, magnesium gluconate, magnesium glycerophosphate, magnesium lactate, magnesium sulphate, any water-soluble magnesium salt, or mixtures thereof.

Under normal conditions, in healthy individuals, only 30%-50% of the magnesium ingested, is absorbed. The absorption takes place throughout the digestive tract, but especially in the distal portion of the duodenum and in the ileum. The absorption is essentially passive. The homeostasis of magnesium is provided primarily by the renal filtration-re-absorption process. In case of elevated intake, the kidney eliminates the excess magnesium; conversely, when the intake declines, renal re-absorption increases. A composition containing the indigestible oligosaccharide significantly increases the intestinal absorption of the magnesium, with simultaneous improvement of the various urinary and plasmic magnesium markers. An increase in calcium intake does not reduce the absorption of magnesium, and vitamin D has practically no influence. By utilisation of a composition comprising the functional ingredient enriched with a magnesium salt, the absorption and the bio-availability is positively influenced.

The present invention further relates to the use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans.

The present invention further relates to the use wherein the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

Furthermore, the present invention relates to the use wherein the indigestible oligosaccharide is administered in a daily dose of between 1 to 20 g/kg body weight, preferably between 2 to 17 g/kg body weight, more preferably between 5 to 15 g/kg body weight.

The present invention relates to the use of an indigestible oligosaccharide in the preparation of a food product for increasing absorption and/or bioavailability of magnesium.

The present invention relates to a food product containing aforementioned composition and said food product is selected from the group consisting of bakery products, snacks, breakfast cereals, cereal bars, dairy products, desserts, confectionery products, dietary supplements and beverages. In fact, the composition can be applied in any food composition wherein indigestible oligosaccharides are compatible with the other ingredients of the food product. Through the normal consumption of food products comprising aforementioned composition, the dietary intake of magnesium is improved, and the absorption is increased.

The present invention relates to the use of indigestible oligosaccharide in the preparation of a pharmaceutical product for increasing absorption and/or bioavailability of magnesium.

Furthermore, the present invention describes a pharmaceutical product comprising the aforementioned composition and said pharmaceutical product is provided with a carrier selected from the group consisting of tablets, lozenges, capsules, suspensions and syrups.

The pharmaceutical product can be administered to healthy persons as a preventive measure for avoiding magnesium deficiency. Healthy persons and especially pregnant women can benefit from the consumption of food products containing the aforementioned composition and additional intake of pharmaceutical products comprising the composition can fortify the effect of the functional ingredient in the food product. On the other hand, the pharmaceutical product can be administered to persons already suffering from a mild to severe magnesium deficiency. In particular, women suffering from osteoporosis or pregnant women might get relieve by applying the pharmaceutical product of the current invention.

In particular, using 10 grams/kg body weight, per day of short-chain fructo-oligosaccharides increases the intestinal absorption of the magnesium by at least 11%, with simultaneous improvement of the various urinary and plasmic magnesium markers.

The current invention has the following advantages:

- The composition comprises a functional ingredient, which can be an indigestible oligosaccharide alone, or it can be enriched with a magnesium salt.
- The composition can be used in the normal diet when being part of a food product.
- The composition can be applied as medicine when it is incorporated in a pharmaceutical product.
- The composition significantly improves the bioavailability of magnesium in humans.
- The composition makes it possible to ameliorate or provide adequate magnesium levels.

The invention is illustrated by way of the following example.

Example.

Study design

12 subjects (= healthy postmenopausal women between 50-70 years and at least 2 years of menopause in the age-group, having a body mass index between 20-27 kg/m² and not involved a hormone replacement therapy (HRT)) received for 35 days short-chain fructo-oligosaccharides (Actilight®) and placebo treatments according to a randomized, double-blind, crossover design. Treatments were separated by a washout period of at least 3 weeks. Both treatments were supplied to volunteers as 5 g powder sachet. Volunteers received 5 g short-chain fructo-oligosaccharides per day for the first 4 days only at lunch and then 10 g/d (5 g at lunch and 5 g at dinner) in order to obtain the best adaptation.

During the first 23 days, volunteers were asked to maintain their normal food intake. They were not allowed to eat oligosaccharides containing food products, or mineral and vitamin supplements. During the final 10-12 days, all subjects consumed a controlled diet in the metabolic unit (2100 Kcal/d, 15% protein, 35 % fat, and 50 % carbohydrates). This diet supplied daily about 900 mg Ca, 250 mg Mg and 12 g dietary fibers. On day 28, the volunteers received at lunch the isotope dose (87.5 mg of ^{25}Mg in 100 ml aromatized water), and 40 radio-opaque pellets as fecal marker. Twenty-four hours urine was collected for two successive days. Fecal collections started immediately after intake of the stable isotopes and lasted 5-7 days. Feces were collected every 24h, frozen and allowed to a radiography in order to count radio-opaque.

During the 5-7 days following the stable isotope administration, standard meals were offered to volunteers. Leftover food was collected to determine net food consumption. Accordingly, net Mg intake was calculated.

Stable isotope analysis.

All feces, collected for 5-7 days after isotope administration, were weighted, X-rayed, pooled and thoroughly homogenized. Subsequently, the samples were freeze-dried and the dry weight was determined. Sub-samples (0.25 g) were ashed at 500°C for 10 hours. The ash was dissolved in 0.5 ml 14 M-HNO₃ and 0.2 ml H₂O₂ and heated at 110°C for 2 hours. The temperature was then increased to 130°C until all the acid was evaporated. 10 ml of 0.16 MHNO₃ was added to all samples.

Total Mg contents in diets, feces, plasma, erythrocytes and urine were measured by flame atomic absorption spectrometry (Perkin-Elmer 560, Paris, France). For Mg stable isotope measurements, Mg concentrations were first adjusted to 40-80 µg/l in HNO₃ (= 0.14 mol/L) prior to isotope ratio analysis. $^{25}\text{Mg}^{26}\text{Mg}$ and $^{25}\text{Mg}^{24}\text{Mg}$ ratios were determined in basal (before isotope administration) and in 5 to 7-day feces samples by inductive

coupled plasma mass spectrometer (ICP/MS). The $^{25}\text{Mg}/^{26}\text{Mg}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ ratios were also determined in plasma and urine collected before and after ^{25}Mg isotope administration. The mass spectrometer settings and plasma conditions were optimized with a solution of 10 μg indium/l.

The instrument operating conditions were as follows: radio frequency (RF) generator 27.12 MHz, forward RF power 1350 W, reflected RF power < 3 W, outer Ar flow rate 14 l/min, intermediate Ar flow rate 0.7 l/min, nebulizer Ar flow rate 0.76 l/min, mass resolution 0.9 Da, at 10 % of peak height.

Calculations

Intestinal Mg absorption (%) was calculated as follows:

$$100 * \frac{(\text{Administered isotope amount} - \text{non-absorbed isotopes corrected for fecal marker})}{\text{Administered isotope amount}}$$

“Non absorbed isotopes” corrected for fecal marker excretion, was obtained as follows:

$$\frac{\text{Non-absorbed isotopes} * \text{Number of excreted pellets}}{\text{Number of total ingested pellets}}$$

Non-absorbed isotope was calculated as follows:

$$\frac{\text{total (Mg) in feces} * E * \text{natural abundance of } ^{25}\text{Mg}}{1 + (E * \text{natural abundance of } ^{25}\text{Mg})}$$

Where E (isotope enrichment) was calculated as follows

$$\frac{\text{MIR} - \text{bIR}}{\text{bIR}}$$

Where bIR was the basal isotopic ratio before isotope administration, and mIR was the measured isotopic ratio after isotope administration.

Mg status evaluation

Blood and urine samples were collected at d0, d28 of each experimental period. Plasma was separated for total Mg determination, and an aliquot of erythrocytes was sampled for Mg content determination. The 24-hours urine was also collected during two successive 24-hour periods after isotope administration. Each 24-hours urine volume was measured and sub-samples were acidified with 16M-HNO₃ (1 volume acid for 100 volume urine) and frozen until analysis.

Total Mg content in plasma and urine was measured by flame atomic absorption spectrometry (Perkin Elmer 560), after an appropriate sample dilution in 0.1% lanthanum, at 285 nm, using an acetylene-air flame.

Results

Results were considered statistically different when p-value was ≤ 0.05 .

Mg intake assessment

Mg intake was about 250 mg/d through the study, which is below the safe and adequate dietary intake for Mg. The Mg intake assessed at the middle and at the end period for both treatments were not different and amounted 250 mg/d. Estimated daily fiber intakes averaged 14.3 and 17.2 g at the middle of each period for short-chain fructooligosaccharides treatments and placebo, respectively. However, the daily fiber intake for the controlled diet periods amounted to about 12 g/d for both treatments.

Feces excretion

The fecal excretion was significantly different both in wet (85.7 \pm 27.6 vs 120.1 \pm 37.4 g) and dry (18.5 \pm 4.2 vs 23.9 \pm 5.1 g) weights, for placebo and short-chain fructooligosaccharides treatments, respectively. Fecal pH was similar, 7.48 \pm 0.69 vs 7.40 \pm

0.48, for placebo and short-chain fructo-oligosaccharides periods, respectively.

Intestinal Mg absorption

Values of intestinal Mg absorption are given in the table 1.

Table 1: Effect of short-chain fructo-oligosaccharides intake on intestinal Mg absorption in postmenopausal women

	Administered ²⁵ Mg (mg)	fecal ²⁵ Mg excretion (mg)	²⁵ Mg (mg) absorption (%)	Pellet excretion (%)
Placebo	87.5	59.7 ± 4.9	30.2 ± 4.5	98.2 ± 2.5
Sc-FOS	87.5	57.1 ± 6.0	33.9 ± 7.2	98.2 ± 3.7
P (one sided)		0.007	0.007	>0.100

Results are expressed as mean ± SD.

Sc-FOS = short-chain fructo-oligosaccharides

The intestinal Mg absorption averaged 30.2±4.5% during placebo treatment and increased to 33.9±7.2% when volunteers consumed short-chain fructo-oligosaccharides (+11 %, p<0.05).

The effect short-chain fructo-oligosaccharides on urine and plasma ²⁵Mg enrichment is shown in table 2.

Table 2: Effect of sc-FOS intake on urine and plasma ^{25}Mg enrichments and on ^{25}Mg urinary excretion per 24 hours in postmenopausal women¹

	Plasma ^{25}Mg Enrichment (%)	Urine (day 1) ^{25}Mg excretion mg	Urine (day 1) ^{25}Mg Enrichment (%)	Urine (day 2) ^{25}Mg excretion (mg)	Urine (day 2) ^{25}Mg Enrichment (%)
Placebo	10.6 ± 1.6	13.7 ± 2.3	1.05 ± 0.4	9.6 ± 1.4	0.70 ± 0.22
Sc-FOS	11.4 ± 1.7	14.8 ± 2.6	1.24 ± 0.50	10.2 ± 1.6	0.90 ± 0.29
P (one sided)	0.012	0.040	0.079	0.042	0.046

1) Results are expressed as mean \pm SD.

2) Sc-FOS = short-chain fructo-oligosaccharides

Plasma ^{25}Mg enrichment, measured 24 h after ^{25}Mg administration, was increased by 8% ($p < 0.05$) during short-chain fructo-oligosaccharides treatment. Moreover, during short-chain fructo-oligosaccharides treatment the urinary ^{25}Mg enrichment was significantly increased by 8% on the first day and by 6% on the second day ($p < 0.05$). In addition, the amount of ^{25}Mg excreted in the urine was increased by 18% on the first day ($p = 0.08$) and by 29% on the second day ($p < 0.05$).

Total Mg contents in blood and urine

Results are displayed in Table 3.

Table 3: Effect of sc-FOS intake on Mg status and total urinary Mg excretion in postmenopausal women

	Plasma Mg			Red Blood cells Mg		
	d0	d28	d28 - d0	d0	d28	28-d0
	mg /l	mg /l	mg /l	mg /l	mg /l	mg /l
Placebo	21.8±2.6	21.3±2.1	-0.5±2.7	52.7±3.6	53.2±4.6	0.60±2.92
Sc-FOS	20.7±2.3	22.0±1.7	1.3±2.1	52.5±4.4	53.5±4.1	1.00±4.89
P(one sided)	>0.100	>0.100	0.014	>0.100	>0.100	>0.100

	Urinary total Mg excretion					
	mg Mg/mmol creatinine			mg Mg /24 hours		
	d0	d28	d28 - d0	d29	d30	(d29+d30)/2
Placebo	6.82±3.06	10.3±2.6	3.44±4.01	76.3±25.4	72.4±18.2	74.4±21.1
Sc-FOS	8.04±4.19	11.2±4.1	3.12±5.22	82.5±23.7	88.2±22.0	85.4±19.9
P (one sided)	>0.100	>0.100	>0.100	>0.100	0.010	0.019

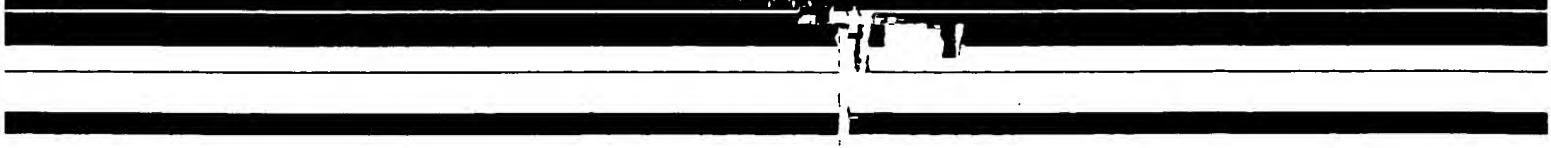
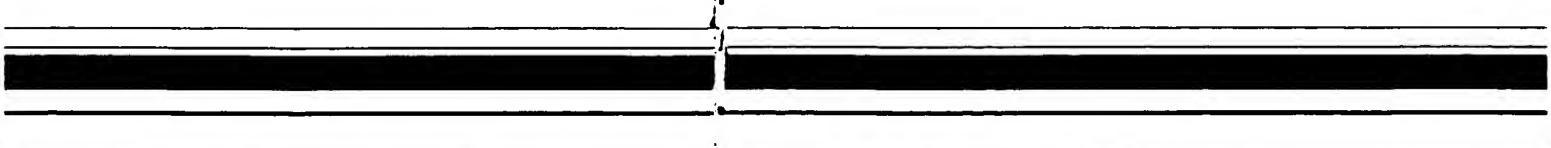
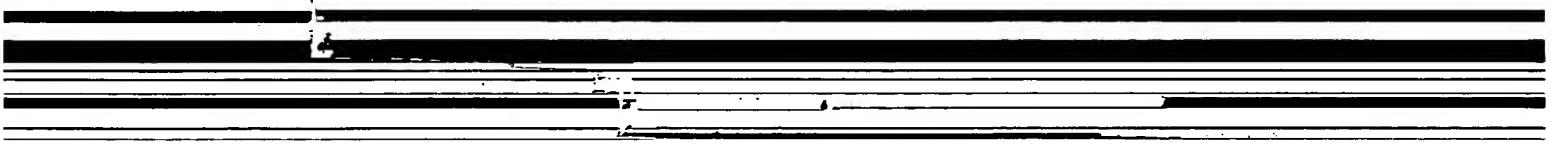
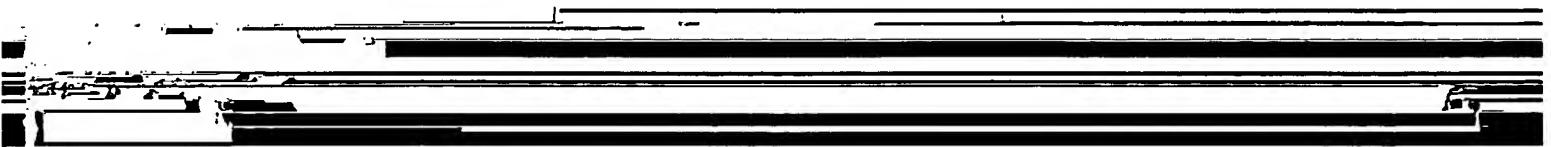
1) Results are expressed as mean ± SD

2) Sc-FOS = short-chain fructo-oligosaccharides.

Total plasma and red blood cell Mg levels were statistically unchanged before and after treatments. Nevertheless, the variation in plasma Mg level between d0 and d28, showed a strong trend to increase ($p=0.0532$) when volunteers were under short-chain fructo-oligosaccharides treatment. Urinary Mg level measured on urine spots at the beginning and 28d after short-chain fructo-oligosaccharides or placebo introduction, and expressed as mg Mg/mmol creatinine was not altered by intake of the test product. However, total Mg urinary excretion measured over 2 successive days, after 4 weeks of treatment, was significantly increased during short-chain fructo-oligosaccharides treatment compared to placebo.

Claims

1. A composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium.
2. A composition according to claim 1 characterised in that the functional ingredient is present in an amount of between 1% w/w to 99% w/w, preferably between 2% w/w to 90% w/w, more preferably between 5% w/w to 80% w/w, most preferably between 20% w/w to 50% w/w.



oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

7. Use according to claim 5 or 6 characterised in that the indigestible oligosaccharide is administered in a daily dose of between 1 to 20 g/kg body weight, preferably between 2 to 17 g/kg body weight, more preferably between 5 to 15 g/kg body weight.
8. Use of indigestible oligosaccharide in the preparation of a food product for increasing absorption and/or bioavailability of magnesium.
9. A food product characterised in that said food product is comprising the composition according to anyone of claim 1 to 4 and said food product is selected from the group consisting of bakery products, snacks, breakfast cereals, cereal bars, dairy products, desserts, confectionery products, dietary supplements and beverages.
10. Use of indigestible oligosaccharide in the preparation of a pharmaceutical product for increasing absorption and/or bioavailability of magnesium.
11. A pharmaceutical product characterised in that said pharmaceutical product is comprising the composition according to anyone of claim 1 to 4 and said pharmaceutical product is provided with a carrier selected from the group consisting of tablets, lozenges, capsules, suspensions and syrups.

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/EP 01/13437

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7	A23L1/304	A61K31/70
		A61K47/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
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BIOSIS, EPO-Internal, PAJ, WPI Data, FSTA, CHEM ABS Data
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C. DOCUMENTS CONSIDERED TO BE RELEVANT
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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SAKO TOMOYUKI ET AL: "Recent progress on research and applications of non-digestible galacto-oligosaccharides." INTERNATIONAL DAIRY JOURNAL, vol. 9, no. 1, 1999, pages 69-80, XP000998503 ISSN: 0958-6946 cited in the application page 76 -page 78</p> <p>---</p> <p style="text-align: center;">-/-</p>	1,2,4-11

<input checked="" type="checkbox"/>	Further documents are listed in the continuation of box C.
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<input checked="" type="checkbox"/>	Patent family members are listed in annex.
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* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the International search report
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18 March 2002	22/03/2002
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer
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Lepretre, F

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/EP 01/13437

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZIEMER C J ET AL: "AN OVERVIEW OF PROBIOTICS, PREBIOTICS AND SYNBiotics IN THE FUNCTIONAL FOOD CONCEPT: PERSPECTIVES AND FUTURE STRATEGIES" INTERNATIONAL DAIRY JOURNAL, ELSEVIER APPLIED SCIENCE, BARKING, GB, vol. 8, May 1998 (1998-05), pages 473-479, XP000972201 ISSN: 0958-6946 cited in the application page 475 -page 477 ---	1, 4, 9, 11
X	COLLINS M D ET AL: "PROBIOTICS, PREBIOTICS, AND SYNBiotics: APPROACHES FOR MODULATING THE MICROBIAL ECOLOGY OF THE GUT1,2" AMERICAN JOURNAL OF CLINICAL NUTRITION, BETHESDA, MD, US, vol. 69, no. SUPPL, May 1999 (1999-05), pages 1052S-1057S, XP000972408 ISSN: 0002-9165 cited in the application page 1053 -page 1054 ---	1, 4, 9, 11
X	PATENT ABSTRACTS OF JAPAN vol. 016, no. 419 (C-0981), 3 September 1992 (1992-09-03) & JP 04 144663 A (NOURINSUISANSHIYOU CHIYUUGOKU NOUGIYOU SHIKENJIYOUUCHIYOU), 19 May 1992 (1992-05-19) abstract ---	1, 4, 9, 11
A	US 4 959 222 A (NADLAND KARL J ET AL) 25 September 1990 (1990-09-25) claims -----	1-11

INTERNATIONAL SEARCH REPORT

Int'l Application No

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Patent document cited in search report		Publication date		Patent family member(s)		Publication date
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			SE	8803409 A		29-03-1989

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(71) Applicant (for all designated States except US): BEGHIN MEIJI [FR/FR]; 14, boulevard du Général Leclerc, F-92572 Neuilly-sur-Seine Cedex (FR).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

(72) Inventors; and

(75) Inventors/Applicants (for US only): BROUNS, Fredericus, Johannes, Petrus, Henricus [NL/NL]; Sibberkerstraat 38, NL-6301 Valkenberg (NL). BORNET, Francis, Robert, Joseph [FR/FR]; 122, avenue du Maine, F-75014 Paris (FR). COUDRAY, Charles [FR/FR]; 15 rue Alexandre Geneix, F-63100 Clermont-Ferrand (FR). RAYSSIGUIER, Yves, Georges [FR/FR]; 9, rue Jacques Prevert, Saulzet Le Chaud, F-63540 Romagnat (FR).

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(54) Title: INDIGESTIBLE OLIGOSACCHARIDES AND MAGNESIUM ABSORPTION IN HUMANS

(57) Abstract: The present invention relates to a composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium. The indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof. Furthermore, the use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans is disclosed.

Indigestible oligosaccharides and magnesium absorption in humans

Technical field

The present invention is in the field of human nutrition and health. Specifically it relates to a method of increasing absorption and bioavailability of magnesium. Indigestible oligosaccharides are shown to have this effect when added in effective amounts to normal food.

Background of the invention

Magnesium is an essential dietary element that plays an important role in the body. It is acting as a cofactor in many enzymatic reactions, including glucose use, the synthesis of fat, proteins and nucleic acids, the metabolism of adenosine triphosphate, muscle contraction and some membrane transport systems. It is active in all the major metabolic processes involved in the body's defences, stimulating growth and immunity. It has multiple effects and properties like anti-stress, antiallergenic, anti-inflammatory, radioprotective, and acting as a thermal regulator.

Tucker K. et. al in Journal of Bone Mineral Research 10, (1995), S466 shows the role of magnesium in the prevention of osteoporosis by diminution of bone. Durlach J. et. al in Magnesium Research 12, 1. (1999) pages 57-61 demonstrates the importance of magnesium in the reduction of risk of coronary disease. Similarly, Mizushima S., et. al. in J. Hum. Hypertens. 12(7). (1998) pages 447-53 shows that magnesium is involved in the reduction of hypertension. Furthermore, Djurhuus MS, et.al. in Diabetes Care, Apr. 22(4) (1999) pages 546-54 demonstrates that magnesium is involved in the body's ability to secrete insulin and to reduce insulin resistance in diabetics.

T. Sako et al. in International Dairy Journal 9 (1999), pages 69-80 shows that fructans such as inulin and fructo-oligosaccharides effect calcium and magnesium absorption in the rat intestine. However, it is further indicated that these results are not supported by human studies. In fact, up to now the contrary has been proven and compositions of galacto-oligosaccharides, inuline, or fructo-oligosaccharides do not lead to improvement in calcium or iron absorption in young male volunteers.

C.J. Ziemer et. al. in Int. Dairy Journal 8 (1998) pages 473 – 479 describes that fructo-oligosaccharides are able to modify the gut flora composition in favour of bifidobacteria.

E. van den Heuvel et. al. in Am. J. Clin. Nutr. 1998; 67: 445-451 describes that inuline, fructo-oligosaccharides, or galacto-oligosaccharides do not have a negative effect on iron and calcium absorption in young healthy men. However, no positive indication nor any effect on magnesium absorption is disclosed.

M. D. Collins et al. in Am. J. Clin. Nutr. 1999; 69(suppl.): 1052S-1057S provides an overview of how probiotics, prebiotics, and synbiotics may contribute toward nutritional modulation of the gut microecology. However, beneficial aspects of human gut flora still need definitive confirmation.

Currently, in the United States and in Europe, magnesium supplied by food is often below the recommendations. Whereas the recommended intake corresponds to around 6 mg per kg per day, the normal daily intake is only in the order of 4 mg per kg. A large proportion of the population therefore presents a primary, chronic magnesium deficiency, through nutritional insufficiency. Such a chronic magnesium deficiency can result in chronic fatigue, signs of neuromuscular hyper-excitability analogous to those described under latent tetany and hyperventilation syndrome.

Accordingly, there exists a need for improving the nutritional intake of magnesium.

The current invention provides a composition for increasing the absorption and/or bioavailability of magnesium.

Summary of the invention

The present invention relates to a composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium.

The present invention relates to a composition wherein the functional ingredient is present in an amount of between 1% w/w to 99% w/w, preferably between 2% w/w to 90% w/w, more preferably between 5% w/w to 80% w/w, most preferably between 20% w/w to 50% w/w.

The present invention further relates to a composition wherein the functional ingredient is enriched with 1% w/w to 20% w/w magnesium salt, preferably between 3% w/w and 10% w/w magnesium salt.

The composition of the current invention is further characterised in that the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

The present invention further relates to the use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans.

The present invention further relates to the use wherein the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

Furthermore, the present invention relates to the use wherein the indigestible oligosaccharide is administered in a daily dose of between 1 to 20 g/kg body weight, preferably between 2 to 17 g/kg body weight, more preferably between 5 to 15 g/kg body weight.

The present invention relates to the use of indigestible oligosaccharide in the preparation of a food product for increasing absorption and/or bioavailability of magnesium.

The present invention relates to a food product containing aforementioned composition and said food product is selected from the group consisting of bakery products, snacks, breakfast cereals, cereal bars, dairy products, desserts, confectionery products, dietary supplements and beverages.

The present invention relates to the use of indigestible oligosaccharide in the preparation of a pharmaceutical product for increasing absorption and/or bioavailability of magnesium.

Furthermore, the present invention describes a pharmaceutical product comprising the aforementioned composition and said pharmaceutical product is provided with a carrier selected from the group consisting of tablets, lozenges, capsules, suspensions and syrups.

Detailed description of the invention

The present invention relates to a composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium.

So far, it has been demonstrated that in rat intestine, fructans, inulin and fructo-oligosaccharides effect magnesium absorption. However, rats have a greater cecum and a different food matrix than humans and the results related to improved magnesium absorption cannot be transposed straightforward from rats to humans. In fact, it has been indicated that inulin, fructo-oligosaccharides, or galacto-oligosaccharides do not have a negative effect on iron and calcium absorption in young healthy men (E. van den Heuvel et. al. in Am. J. Clin. Nutr. 1998; 67: 445-451). However, no positive indication nor any effect on magnesium absorption is disclosed.

Surprisingly, the current invention demonstrates that an effective amount of indigestible oligosaccharide increases in humans the absorption and/or bioavailability of magnesium.

The effective amount of the functional ingredient is the dose, which is sufficient for obtaining an improved absorption and/or bioavailability of magnesium in humans.

The absorption of magnesium can be measured by stable magnesium isotope analysis with inductive coupled plasma mass spectrometer (ICP/MS).

Indigestible oligosaccharide refers to a carbohydrate moiety that is resistant to endogenous digestion in the human upper digestive tract.

The present invention relates to a composition wherein the functional ingredient is present in an amount of between 1% w/w to 99% w/w, preferably between 2% w/w to 90% w/w, more preferably between 5% w/w to 80% w/w, most preferably between 20% w/w to 50% w/w.

The composition of the current invention is further characterised in that the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides or isomalto-oligosaccharides.

Xylo-oligosaccharides are produced by the enzymic hydrolysis from xylan, which is one of the main components of dietary fibre, and it is a major component of plant hemicellulose.

Galacto-oligosaccharides comprise di-, tri-, tetra-, penta- and hexasaccharides, mainly consisting of galactose as a sugar component, and are formed by the action of β -galactosidase on lactose.

Soybean oligosaccharides are the water-soluble saccharides extracted from soybean whey, a by-product from the production of soy protein. Mainly mixtures of mono-, di-, tri-, and tetrasaccharides with as principal components raffinose and stachyose are obtained.

Gentio-oligosaccharides are polymers in which 6- β -glucopyranosyl-glucose constitute the majority of linkages.

Isomalto-oligosaccharides are mixtures of isomaltose, panose, isomaltotriose, and several other branched oligosaccharides composed of four and five glucose residues.

Fructans are polymers in which fructosyl-fructose linkages constitute the majority of linkages.

Fructo-oligosaccharides are indigestible oligosaccharides that are members of the inuline subclass of fructans. Fructo-oligosaccharides occur in nature in many kinds of plants, including onions, garlic, shallots, wheat, rye, bananas, aspargus, tomatoes, artichokes, dahlia and chicory root. Fructo-oligosaccharides can be produced enzymatically, through chemical techniques or by extraction from natural substances. Short chain fructo-oligosaccharides are composed of one to three fructose molecules linked to one molecule of sucrose. Their polymerisation degree (DP) is not higher than 6, and they can be synthesised from sucrose through the use of transfructosylating enzymes. Treatment of sucrose with these transfructosylating enzymes results in a mixture of

fructo-oligosaccharides containing 2, 3 or 4 fructose units, such as 1-kestose, nystose, and fructosyl-nystose.

The current invention surprisingly demonstrates that an effective amount of these short chain fructo-oligosaccharides increases in humans the absorption and/or bioavailability of magnesium.

The present invention further relates to a composition wherein the functional ingredient is enriched with 1% w/w to 20% w/w magnesium salt, preferably between 3% w/w and 10% w/w magnesium salt.

The magnesium salt can be magnesium carbonate, magnesium citrate, magnesium gluconate, magnesium glycerophosphate, magnesium lactate, magnesium sulphate, any water-soluble magnesium salt, or mixtures thereof.

Under normal conditions, in healthy individuals, only 30%-50% of the magnesium ingested, is absorbed. The absorption takes place throughout the digestive tract, but especially in the distal portion of the duodenum and in the ileum. The absorption is essentially passive. The homeostasis of magnesium is provided primarily by the renal filtration-re-absorption process. In case of elevated intake, the kidney eliminates the excess magnesium; conversely, when the intake declines, renal re-absorption increases. A composition containing the indigestible oligosaccharide significantly increases the intestinal absorption of the magnesium, with simultaneous improvement of the various urinary and plasmic magnesium markers. An increase in calcium intake does not reduce the absorption of magnesium, and vitamin D has practically no influence. By utilisation of a composition comprising the functional ingredient enriched with a magnesium salt, the absorption and the bio-availability is positively influenced.

The present invention further relates to the use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans.

The present invention further relates to the use wherein the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

Furthermore, the present invention relates to the use wherein the indigestible oligosaccharide is administered in a daily dose of between 1 to 20 g/kg body weight, preferably between 2 to 17 g/kg body weight, more preferably between 5 to 15 g/kg body weight.

The present invention relates to the use of an indigestible oligosaccharide in the preparation of a food product for increasing absorption and/or bioavailability of magnesium.

The present invention relates to a food product containing aforementioned composition and said food product is selected from the group consisting of bakery products, snacks, breakfast cereals, cereal bars, dairy products, desserts, confectionery products, dietary supplements and beverages. In fact, the composition can be applied in any food composition wherein indigestible oligosaccharides are compatible with the other ingredients of the food product. Through the normal consumption of food products comprising aforementioned composition, the dietary intake of magnesium is improved, and the absorption is increased.

The present invention relates to the use of indigestible oligosaccharide in the preparation of a pharmaceutical product for increasing absorption and/or bioavailability of magnesium.

Furthermore, the present invention describes a pharmaceutical product comprising the aforementioned composition and said pharmaceutical product is provided with a carrier selected from the group consisting of tablets, lozenges, capsules, suspensions and syrups.

The pharmaceutical product can be administered to healthy persons as a preventive measure for avoiding magnesium deficiency. Healthy persons and especially pregnant women can benefit from the consumption of food products containing the aforementioned composition and additional intake of pharmaceutical products comprising the composition can fortify the effect of the functional ingredient in the food product. On the other hand, the pharmaceutical product can be administered to persons already suffering from a mild to severe magnesium deficiency. In particular, women suffering from osteoporosis or pregnant women might get relieve by applying the pharmaceutical product of the current invention.

In particular, using 10 grams/kg body weight, per day of short-chain fructo-oligosaccharides increases the intestinal absorption of the magnesium by at least 11%, with simultaneous improvement of the various urinary and plasmic magnesium markers.

The current invention has the following advantages:

- The composition comprises a functional ingredient, which can be an indigestible oligosaccharide alone, or it can be enriched with a magnesium salt.
- The composition can be used in the normal diet when being part of a food product.
- The composition can be applied as medicine when it is incorporated in a pharmaceutical product.
- The composition significantly improves the bioavailability of magnesium in humans.
- The composition makes it possible to ameliorate or provide adequate magnesium levels.

The invention is illustrated by way of the following example.

Example.

Study design

12 subjects (= healthy postmenopausal women between 50-70 years and at least 2 years of menopause in the age-group, having a body mass index between 20-27 kg/m² and not involved a hormone replacement therapy (HRT)) received for 35 days short-chain fructo-oligosaccharides (Actilight®) and placebo treatments according to a randomized, double-blind, crossover design. Treatments were separated by a washout period of at least 3 weeks. Both treatments were supplied to volunteers as 5 g powder sachet. Volunteers received 5 g short-chain fructo-oligosaccharides per day for the first 4 days only at lunch and then 10 g/d (5 g at lunch and 5 g at dinner) in order to obtain the best adaptation.

During the first 23 days, volunteers were asked to maintain their normal food intake. They were not allowed to eat oligosaccharides containing food products, or mineral and vitamin supplements. During the final 10-12 days, all subjects consumed a controlled diet in the metabolic unit (2100 Kcal/d, 15% protein, 35 % fat, and 50 % carbohydrates). This diet supplied daily about 900 mg Ca, 250 mg Mg and 12 g dietary fibers. On day 28, the volunteers received at lunch the isotope dose (87.5 mg of ^{25}Mg in 100 ml aromatized water), and 40 radio-opaque pellets as fecal marker. Twenty-four hours urine was collected for two successive days. Fecal collections started immediately after intake of the stable isotopes and lasted 5-7 days. Feces were collected every 24h, frozen and allowed to a radiography in order to count radio-opaque.

During the 5-7 days following the stable isotope administration, standard meals were offered to volunteers. Leftover food was collected to determine net food consumption. Accordingly, net Mg intake was calculated.

Stable isotope analysis.

All feces, collected for 5-7 days after isotope administration, were weighted, X-rayed, pooled and thoroughly homogenized. Subsequently, the samples were freeze-dried and the dry weight was determined. Sub-samples (0.25 g) were ashed at 500°C for 10 hours. The ash was dissolved in 0.5 ml 14 M-HNO₃ and 0.2 ml H₂O₂ and heated at 110°C for 2 hours. The temperature was then increased to 130°C until all the acid was evaporated. 10 ml of 0.16 MHNO₃ was added to all samples.

Total Mg contents in diets, feces, plasma, erythrocytes and urine were measured by flame atomic absorption spectrometry (Perkin-Elmer 560, Paris, France). For Mg stable isotope measurements, Mg concentrations were first adjusted to 40-80 µg/l in HNO₃ (= 0.14 mol/L) prior to isotope ratio analysis. $^{25}\text{Mg}/^{26}\text{Mg}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ ratios were determined in basal (before isotope administration) and in 5 to 7-day feces samples by inductive

coupled plasma mass spectrometer (ICP/MS). The $^{25}\text{Mg}/^{26}\text{Mg}$ and $^{25}\text{Mg}/^{24}\text{Mg}$ ratios were also determined in plasma and urine collected before and after ^{25}Mg isotope administration. The mass spectrometer settings and plasma conditions were optimized with a solution of 10 μg indium/l.

The instrument operating conditions were as follows: radio frequency (RF) generator 27.12 MHz, forward RF power 1350 W, reflected RF power < 3 W, outer Ar flow rate 14 l/min, intermediate Ar flow rate 0.7 l/min, nebulizer Ar flow rate 0.76 l/min, mass resolution 0.9 Da, at 10 % of peak height.

Calculations

Intestinal Mg absorption (%) was calculated as follows:

$$\frac{100 * (\text{Administered isotope amount} - \text{non-absorbed isotopes corrected for fecal marker})}{\text{Administered isotope amount}}$$

“Non absorbed isotopes” corrected for fecal marker excretion, was obtained as follows:

$$\frac{\text{Non-absorbed isotopes} * \text{Number of excreted pellets}}{\text{Number of total ingested pellets}}$$

Non-absorbed isotope was calculated as follows:

$$\frac{\text{total (Mg) in feces} * E * \text{natural abundance of } ^{25}\text{Mg}}{1 + (E * \text{natural abundance of } ^{25}\text{Mg})}$$

Where E (isotope enrichment) was calculated as follows

$$\frac{\text{MIR} - \text{bIR}}{\text{bIR}}$$

Where bIR was the basal isotopic ratio before isotope administration, and mIR was the measured isotopic ratio after isotope administration.

Mg status evaluation

Blood and urine samples were collected at d0, d28 of each experimental period. Plasma was separated for total Mg determination, and an aliquot of erythrocytes was sampled for Mg content determination. The 24-hours urine was also collected during two successive 24-hour periods after isotope administration. Each 24-hours urine volume was measured and sub-samples were acidified with 16M-HNO₃ (1 volume acid for 100 volume urine) and frozen until analysis.

Total Mg content in plasma and urine was measured by flame atomic absorption spectrometry (Perkin Elmer 560), after an appropriate sample dilution in 0.1% lanthanum, at 285 nm, using an acetylene-air flame.

Results

Results were considered statistically different when p-value was ≤ 0.05 .

Mg intake assessment

Mg intake was about 250 mg/d through the study, which is below the safe and adequate dietary intake for Mg. The Mg intake assessed at the middle and at the end period for both treatments were not different and amounted 250 mg/d. Estimated daily fiber intakes averaged 14.3 and 17.2 g at the middle of each period for short-chain fructooligosaccharides treatments and placebo, respectively. However, the daily fiber intake for the controlled diet periods amounted to about 12 g/d for both treatments.

Feces excretion

The fecal excretion was significantly different both in wet (85.7 \pm 27.6 vs 120.1 \pm 37.4 g) and dry (18.5 \pm 4.2 vs 23.9 \pm 5.1 g) weights, for placebo and short-chain fructooligosaccharides treatments, respectively. Fecal pH was similar, 7.48 \pm 0.69 vs 7.40 \pm

0.48, for placebo and short-chain fructo-oligosaccharides periods, respectively.

Intestinal Mg absorption

Values of intestinal Mg absorption are given in the table 1.

Table 1: Effect of short-chain fructo-oligosaccharides intake on intestinal Mg absorption in postmenopausal women

	Administered	fecal ^{25}Mg	^{25}Mg (mg)	Pellet
	^{25}Mg (mg)	excretion (mg)	absorption (%)	excretion (%)
Placebo	87.5	59.7 \pm 4.9	30.2 \pm 4.5	98.2 \pm 2.5
Sc-FOS	87.5	57.1 \pm 6.0	33.9 \pm 7.2	98.2 \pm 3.7
P (one sided)		0.007	0.007	>0.100

Results are expressed as mean \pm SD.

Sc-FOS = short-chain fructo-oligosaccharides

The intestinal Mg absorption averaged 30.2 \pm 4.5% during placebo treatment and increased to 33.9 \pm 7.2% when volunteers consumed short-chain fructo-oligosaccharides (+11 %, p<0.05).

The effect short-chain fructo-oligosaccharides on urine and plasma ^{25}Mg enrichment is shown in table 2.

Table 2: Effect of sc-FOS intake on urine and plasma ^{25}Mg enrichments and on ^{25}Mg urinary excretion per 24 hours in postmenopausal women¹

	Plasma ^{25}Mg Enrichment (%)	Urine (day 1) ^{25}Mg excretion mg	^{25}Mg Enrichment (%)	^{25}Mg excretion (mg)	Urine (day 2) ^{25}Mg Enrichment (%)
Placebo	10.6 ± 1.6	13.7 ± 2.3	1.05 ± 0.4	9.6 ± 1.4	0.70 ± 0.22
Sc-FOS	11.4 ± 1.7	14.8 ± 2.6	1.24 ± 0.50	10.2 ± 1.6	0.90 ± 0.29
P (one sided)	0.012	0.040	0.079	0.042	0.046

1) Results are expressed as mean \pm SD.

2) Sc-FOS = short-chain fructo-oligosaccharides

Plasma ^{25}Mg enrichment, measured 24 h after ^{25}Mg administration, was increased by 8% ($p < 0.05$) during short-chain fructo-oligosaccharides treatment. Moreover, during short-chain fructo-oligosaccharides treatment the urinary ^{25}Mg enrichment was significantly increased by 8% on the first day and by 6% on the second day ($p < 0.05$). In addition, the amount of ^{25}Mg excreted in the urine was increased by 18% on the first day ($p = 0.08$) and by 29% on the second day ($p < 0.05$).

Total Mg contents in blood and urine

Results are displayed in Table 3.

Table 3: Effect of sc-FOS intake on Mg status and total urinary Mg excretion in postmenopausal women

	Plasma Mg			Red Blood cells Mg		
	d0 mg/l	d28 mg/l	d28 - d0 mg/l	d0 mg/l	d28 mg/l	28-d0 mg/l
Placebo	21.8±2.6	21.3±2.1	-0.5±2.7	52.7±3.6	53.2±4.6	0.60±2.92
Sc-FOS	20.7±2.3	22.0±1.7	1.3±2.1	52.5±4.4	53.5±4.1	1.00±4.89
P(one sided)	>0.100	>0.100	0.014	>0.100	>0.100	>0.100

	Urinary total Mg excretion					
	mg Mg/mmol creatinine			mg Mg /24 hours		
	d0	d28	d28 - d0	d29	d30	(d29+d30)/2
Placebo	6.82±3.06	10.3±2.6	3.44±4.01	76.3±25.4	72.4±18.2	74.4±21.1
Sc-FOS	8.04±4.19	11.2±4.1	3.12±5.22	82.5±23.7	88.2±22.0	85.4±19.9
P (one sided)	>0.100	>0.100	>0.100	>0.100	0.010	0.019

1) Results are expressed as mean ± SD

2) Sc-FOS = short-chain fructo-oligosaccharides.

Total plasma and red blood cell Mg levels were statistically unchanged before and after treatments. Nevertheless, the variation in plasma Mg level between d0 and d28, showed a strong trend to increase ($p=0.0532$) when volunteers were under short-chain fructo-oligosaccharides treatment. Urinary Mg level measured on urine spots at the beginning and 28d after short-chain fructo-oligosaccharides or placebo introduction, and expressed as mg Mg/mmol creatinine was not altered by intake of the test product. However, total Mg urinary excretion measured over 2 successive days, after 4 weeks of treatment, was significantly increased during short-chain fructo-oligosaccharides treatment compared to placebo.

Claims

1. A composition comprising as functional ingredient an effective amount of an indigestible oligosaccharide for increasing in humans the absorption and/or bioavailability of magnesium.
2. A composition according to claim 1 characterised in that the functional ingredient is present in an amount of between 1% w/w to 99% w/w, preferably between 2% w/w to 90% w/w, more preferably between 5% w/w to 80% w/w, most preferably between 20% w/w to 50% w/w.
3. A composition according to claim 1 or 2 characterised in that the functional ingredient is enriched with 1% w/w to 20% w/w magnesium salt, preferably between 3% w/w and 10% w/w magnesium salt.
4. A composition according to anyone of claim 1 to 3 characterised in that the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.
5. Use of indigestible oligosaccharide for increasing the absorption and/or bioavailability of magnesium in humans.
6. Use according to claim 5 characterised in that the indigestible oligosaccharide is selected from the group consisting of xylo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, gentio-oligosaccharides, isomalto-oligosaccharides, fructans, fructo-oligosaccharides, short chain fructo-

oligosaccharides, and mixtures thereof, preferably short chain fructo-oligosaccharides.

7. Use according to claim 5 or 6 characterised in that the indigestible oligosaccharide is administered in a daily dose of between 1 to 20 g/kg body weight, preferably between 2 to 17 g/kg body weight, more preferably between 5 to 15 g/kg body weight.
8. Use of indigestible oligosaccharide in the preparation of a food product for increasing absorption and/or bioavailability of magnesium.
9. A food product characterised in that said food product is comprising the composition according to anyone of claim 1 to 4 and said food product is selected from the group consisting of bakery products, snacks, breakfast cereals, cereal bars, dairy products, desserts, confectionery products, dietary supplements and beverages.
10. Use of indigestible oligosaccharide in the preparation of a pharmaceutical product for increasing absorption and/or bioavailability of magnesium.
11. A pharmaceutical product characterised in that said pharmaceutical product is comprising the composition according to anyone of claim 1 to 4 and said pharmaceutical product is provided with a carrier selected from the group consisting of tablets, lozenges, capsules, suspensions and syrups.

INTERNATIONAL SEARCH REPORT

Int	Application No
PCT/EP 01/13437	

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7	A23L1/304	A61K31/70 A61K47/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A61K A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

BIOSIS, EP0-Internal, PAJ, WPI Data, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SAKO TOMOYUKI ET AL: "Recent progress on research and applications of non-digestible galacto-oligosaccharides." INTERNATIONAL DAIRY JOURNAL, vol. 9, no. 1, 1999, pages 69-80, XP000998503 ISSN: 0958-6946 cited in the application page 76 -page 78</p> <p>---</p> <p style="text-align: center;">-/-</p>	1,2,4-11

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed Invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed Invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *a* document member of the same patent family

Date of the actual completion of the International search

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INTERNATIONAL SEARCH REPORT

Int.	SI Application No
PCT/EP 01/13437	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZIEMER C J ET AL: "AN OVERVIEW OF PROBIOTICS, PREBIOTICS AND SYNBiotics IN THE FUNCTIONAL FOOD CONCEPT: PERSPECTIVES AND FUTURE STRATEGIES" INTERNATIONAL DAIRY JOURNAL, ELSEVIER APPLIED SCIENCE, BARKING, GB, vol. 8, May 1998 (1998-05), pages 473-479, XP000972201 ISSN: 0958-6946 cited in the application page 475 -page 477 ---	1,4,9,11
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